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ONCONEPHROLOGY

The epigenetic landscape of renal cancer

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Abstract | The majority of kidney cancers are associated with mutations in the von Hippel-Lindau gene and a small proportion are associated with infrequent mutations in other well characterized tumour-suppressor genes. In the past 15 years, efforts to uncover other key genes involved in renal cancer have identified many genes that are dysregulated or silenced via epigenetic mechanisms, mainly through methylation of promoter CpG islands or dysregulation of specific microRNAs. In addition, the advent of next-generation sequencing has led to the identification of several novel genes that are mutated in renal cancer, such as *PBRM1*, *BAP1* and *SETD2*, which are all involved in histone modification and nucleosome and chromatin remodelling. In this Review, we discuss how altered DNA methylation, microRNA dysregulation and mutations in histone-modifying enzymes disrupt cellular pathways in renal cancers.

microRNAs
Small, noncoding RNAs that regulate gene expression post-transcriptionally by targeting specific mRNAs for inhibition or degradation through complimentary base pairing.

Kidney cancers are common neoplasms; more than 300,000 new patients are diagnosed worldwide each year[1]. Most renal cancers arise from the epithelium of the renal proximal tubule and are sporadic. Renal cell carcinomas (RCCs) are best treated by resection; however, early diagnosis is difficult and metastases are often present before the primary tumour is detected[2]. As with all cancers, researchers hope that the rapidly increasing understanding of the molecular biology of tumour formation and progression will provide the opportunity to develop new therapeutics and facilitate early diagnosis.

Loss of epigenetic regulation is clearly central to the development of renal tumours[3–5]. In the past 10 years, individual research groups and large consortia have used high-density microarrays that can identify single 5'—C—phosphate—G—3' (CpG) dinucleotides throughout the genome to determine DNA regions that are frequently methylated in tumours. Most of this work has focused on promoter region methylation and the associated control of gene expression[4–9]; however, the involvement of DNA methylation in RCC is more complex than first anticipated. Studies on the epigenetic regulation of other regulatory elements such as distal gene enhancers and repressors are now ongoing. The development of new microarrays with enhancer-specific probes and the increasingly affordable methods of methylation-specific genome-wide sequencing made such studies possible[10].

In addition to DNA methylation, microRNAs (miRNAs) also clearly influence RCC development. Studies using miRNA-specific microarrays and RNA sequencing technologies showed that these small RNA molecules have a central role in the normal physiology of renal cells, and their dysregulation is common in a wide range of RCCs. The number of experimentally validated, physiologically relevant miRNAs increases every year and evidence suggests that thousands of functional miRNAs are still to be identified[11]. Although their importance is clear, many questions regarding the interplay between these noncoding RNAs, mRNAs and proteins during normal cellular physiology and cancer development remain to be elucidated.

RCC was one of the first tumour types for which concerted efforts were made to use massively parallel sequencing, at the time (7 years ago) a newly developed technique, to determine mutations in protein-coding regions (exome sequencing) on large numbers of individual tumours[12–14]. These studies produced surprising results. Previous attempts to identify key, frequent mutations in RCC had failed to yield much beyond the well-established mutations in *VHL*[15]. The exome-sequencing projects identified common mutations in genes encoding histone-modifying proteins. Thus, key driving mutations in RCC can influence the epigenetic control of genome-wide gene expression in renal cells. In the past 3 years, the Cancer Genome Atlas (TCGA) project consortium has published multiplatform analyses of large-scale cohorts of renal cancers[4,5]. These studies confirmed the frequent occurrence of histone modifying protein mutations in RCC and attempted to integrate these findings with global methylation and gene expression analysis.

Epigenetic control of gene expression through methylation of promoters and other regulatory elements, regulation of chromatin via histone modification, and

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Key points

- The most common mutations in renal cancers occur in the *VHL* gene; they disrupt many cellular pathways, notably those involved in the hypoxic response
- Other common mutations affect genes encoding proteins involved in histone modification and chromatin remodelling; mutations of these epigenetic modifier genes influence genome-wide gene expression
- Many key pathways involved in physiological renal cell function are disrupted by inappropriate silencing of component genes through methylation of CpG regions in promoters; these disruptions contribute to renal cancer development
- Alteration of the expression of microRNAs (miRNAs) in renal cancers contributes to the disruption of important cellular networks
- In Wilms tumour, a common childhood renal cancer, miRNA maturation and degradation are often disrupted via mutations of key components of the miRNA biogenesis pathway
- Epigenetically disrupted genes in renal cancers are good candidate targets for the development of robust prognostic and diagnostic tools and novel therapeutics

miRNA-mediated control of protein production, are clearly central to normal cell function and their dysregulation can lead to the development of renal cancers. How these pathways interact and are involved in RCC development and progression remains unclear. This Review discusses epigenetic aberrations in renal cancer and their consequences on known and novel cellular pathways.

Epidemiology and genetics of renal cancer

RCCs include clear cell (cc)RCC (~70% of RCCs), papillary (p)RCC (10–15% of RCCs), and chromophobe (ch)RCC (~5% of Rccs)[16]. Renal cancer usually occurs in adults but it can also affect children. The predominant form of childhood kidney cancer, Wilms tumour (~1 in 10,000 children), has a developmental origin. These tumours are associated with high incidences of additional developmental abnormalities[17].

Unlike many other tumours, inactivating mutations of the tumour suppressor genes *TP53* and *RB1* are not frequent in renal cancers. *TP53* is mutated in 11% and *RB1* in <1% of all kidney cancers, whereas another known tumour-suppressor, *CDKN2A*, is mutated in 10% of patients with RCC[18].

Somatic inactivation of *VHL*, which was originally identified in the hereditary cancer syndrome von Hippel-Lindau (VHL) disease[19], is very common in ccRCC (~52% of patients)[4]. The majority of the inactivating events are point mutations[4,15,20,21]. The VHL disease tumour suppressor protein (pVHL) has multiple functions[22], and is best known as a regulator of oxygen and energy sensing via the targeted degradation of the hypoxia-inducible factors (HIF) 1 and HIF2 (REFS 23–27) **[Au: Refs OK?]**. pVHL also regulates key cellular processes including glucose uptake and metabolism, angiogenesis, pH homeostasis, chemotaxis, proliferation and survival, apoptosis, transcription regulation and cellular senescence through both HIF-dependent and HIF-independent mechanisms[22] (FIG. 1). Many of these key cellular processes can be affected by altered epigenetic regulation.

Non-hereditary pRCC can be subcategorised into pRCC type 1 and pRCC type 2 on the basis of histology and molecular profiles[5,16]. The most common genetic aberrations in type 1 pRCC tumours are activating mutations of *MET*, which are found in ~15% of tumours[5,28]. The most common mutations in type 2 pRCC affect *CDKN2A* (~8% of tumours)[5]. Mutations in *FH*, which encodes a citric acid cycle protein, cause the hereditary leiomyomatosis and RCC syndrome, which confers predisposition to type 2 pRCC[29]. *FH* is also mutated in sporadic pRCC at low frequencies[5,30]. Mutations in *SDHB*, which encodes iron-sulphur subunit of complex II, a component of the enzyme that converts succinate to fumarate in the citric acid cycle, are associated with familial RCC[31–33]. The principal mechanism that drives tumour formation following loss of *FH* or *SDH* is the stabilization of HIF, irrespective of the status of *VHL*[34] (FIG. 1).

chRCCs arise from cells in the distal convoluted tubule of the nephron, whereas ccRCCs arise from cells in the proximal convoluted tubule; this difference in origin might explain the marked differences in the genetic and epigenetic makeup of these tumours. The most frequently mutated genes in somatic chRCC are *TP53* (32% of tumours) and *PTEN* (9% of tumours)[28,35].

Wilms tumours are often associated with developmental syndromes such as Wilms tumour, aniridia, genitourinary abnormalities, mental retardation (WAGR) syndrome, and with deletions of chromosome 11[36]. Only around one-third of Wilms tumours have mutations in *WT1* or *PAX6* (two genes that are often encompassed in chromosome 11 deletions) or in other known tumour suppressor genes such as *TP53*, *WTX* or *CTNNB1* (REF. 37). Since 2012, studies have shown that mutations in genes that encode key components of the microRNA synthesis pathway (*DICER1* and *DROSHA*) or mutations in a component of the RNA exosome complex (*DIS3L2*) are frequent in sporadic Wilms tumours[38–40], indicating the importance of epigenetic regulation in the formation of these developmental tumours.

Epigenetics: basic principles

The availability of transcription factors, both via their presence within a cell and the accessibility of their target sequences in the genome, is essential to orchestrate gene expression. Chromatin states dictate genome accessibility; condensed chromatin prevents access of the transcriptional machinery to the naked DNA, whereas relaxed chromatin allows access (FIG. 2). The localized levels of chromatin condensation are regulated by a complex network of histone-modifying proteins, such as histone acetyltransferases (HATs) and histone deacetylases (HDACs), which determine the transcriptional activity of DNA by adding and removing methyl and acetyl groups from specific amino acids on histones[41] (FIG. 2a,b). The addition of a methyl group to DNA cytosines within CpG islands can recruit HDACs and chromatin remodelling complexes **[Au:OK?]** to induce chromatin condensation and thus, gene silencing[42] (FIG. 2b,c). Gene expression can also be regulated via control of the final protein concentration through post-transcriptional (but pre-translational) regulation of expression via the action of miRNAs[43] and other noncoding RNAs, which were considered to be non-functional until recently[44].

Condensed chromatin
Regions of chromatin where nucleosomes are closely packed together preventing transcription.

Relaxed chromatin
Regions of the chromatin where nucleosomes vacate promoter regions allowing access to transcription factors and the transcriptional machinery.

CpG islands
Region of DNA with a high frequency of 5′–C–phosphate–G–3′ (CpG) dinucleotides. These regions are frequent around transcriptional start sites.

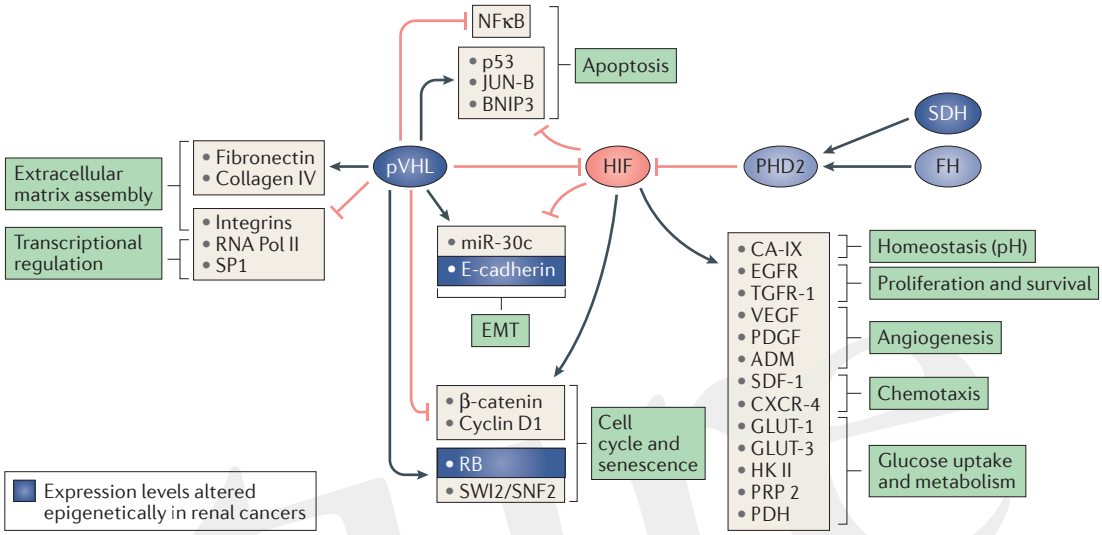

Figure 1 | **Involvement of pVHL in cellular physiology and RCC.** The von Hippel-Lindau disease tumour suppressor protein (pVHL) regulates numerous cellular processes principally through the controlled degradation of hypoxia inducible factor (HIF). Hydroxylation of HIF by hypoxia-inducible factor prolyl hydroxylase 2 (PHD2), enables it to bind to pVHL and be targeted for degradation. This hydroxylation can be inhibited by the accumulation of succinate or fumarate as a consequence of mutations in the genes that encode succinate dehydrogenase (SDH) or fumarate hydrogenase (FH). pVHL also controls cell physiology through multiple HIF-independent mechanisms. These processes are also frequently dysregulated by the epigenetic silencing of regulators, changes in microRNA expression or mutations in chromatin remodelling proteins. ADM, adrenomedullin; BNIP3, BCL2/adenovirus E1B 19 kDa interacting protein 3; CA9, carbonic anhydrase 9; SDF-1, stromal cell-derived factor 1, also known as C-X-C motif chemokine ligand 12; EGFR, epithelial growth factor receptor; EMT, epithelial-to-mesenchymal transition; GLUT-1,3, glucose transporter type 1,3; HK II, hexokinase 2; NFκβ, nuclear factor κβ; PDGF, platelet-derived growth factor; PDH, pyruvate dehydrogenase; PRP 2, primer recognition protein 2, also known as phosphoglycerate kinase 1 (PGK1); RB, retinoblastoma protein; RNA Pol II, RNA polymerase II; RNA pSP1, specificity protein 1 transcription factor; SWI2/SNF, switch/sucrose non-fermentable ATPase (also called transcription activator BRG1 in humans); TGFR-1, transforming growth factor receptor; VEGF, vascular endothelial growth factor.

DNA and histone modifications and miRNA-mediated pathways are the major epigenetic regulatory mechanisms of gene expression. These processes do not change the genomic sequence of a cell but they can be inherited by daughter cells following cell division. These mechanisms are prone to error, often as a consequence of mutations or changes in the expression levels of the genes that encode the complex regulators[45]. As will be discussed below, these epigenetic dysregulations are characteristic of many renal tumours.

DNA methylation in renal cancer

In mammalian genomes methylation takes place predominantly at the cytosine bases that are located on the 5′ side of a guanosine (5 methylcytosine; 5 mC) in a CpG dinucleotide[46,47]. In the past 15 years, most research has focused on identifying changes in the methylation patterns of CpG-rich islands near or in gene promoters, which are clearly directly linked to gene silencing[48,49]. Nevertheless, many other genomic regions also have differential (tissue-specific), or aberrant (cancer) CpG methylation patterns[50–54]. Here, we will discuss the well-defined relationship between tumour specific CpG island methylation and gene silencing.

Promoter methylation

The first cancer-specific epigenetic abnormality identified was a genome-wide reduction in CpG methylation[55]. This global hypomethylation results in genomic instability that drives tumour formation[56,57]. In addition to global hypomethylation, tumour genomes undergo evolutionary selective pressure, which results in tumour suppressor gene silencing through localized promoter hypermethylation. The *RB1* tumour suppressor was the first gene to be identified as silenced in tumours by this mechanism[58]. Many key tumour suppressor genes such as *CDKN2A, TP53, MLH1* and *CDH1* are also commonly inactivated in cancer by promoter methylation[59].

In ~20% of ccRCC[4,60] and ~7% of pRCC[5], a high percentage of CpG islands are methylated. These tumours, which have a CpG island methylator phenotype (CIMP)[61] are aggressive and have increased glycolytic activity. Moreover, CIMP pRCC tumours are associated with poor overall survival[5]. The presence of methyl groups in specific DNA regions depends on DNA methyltrasferase (DNMT) 1, DNMT3A and DNMT3B, which are commonly overexpressed in cancers[62]. These enzymes and other methyl-CpG-binding proteins, such as MBD2, MBD3 and MeCP2, recruit HDACs and other histone-modifying proteins to the methylated promoter region[62,63], which deacetylates histones 3 and 4 and induces chromatin condensation and gene silencing[64].

5 mC can be oxidised to 5-hydroxylmethylcytosine (5hmC) by members of the methylcytosine dioxygenase ten eleven translocation (TET) protein family (TET1, TET2 and TET3)[65–67]. Many differentiated tissues

CpG island methylator phenotype
(CIMP). Tumour phenotype that is characterized by widespread and elevated levels of CpG island methylation. This phenotype represents a clinically and aetiologically distinct group **[Au:OK?]**.

accumulate high levels of 5hmC, which might be unique epigenetic marks and could regulate gene expression[68]. Genome-wide 5hmC levels are markedly reduced in ccRCC tissue compared to adjacent, non-malignant tissue[69]. The precise mechanism of 5hmC loss and its role in malignancy are yet to be elucidated but this epigenetic mark might be a useful biomarker for ccRCC. The precise function of 5hmC and its signal transduction are also not yet known; however, the accumulation of 5hmC upstream of regulatory gene regions suggests a role for this modification in transcriptional regulation[70].

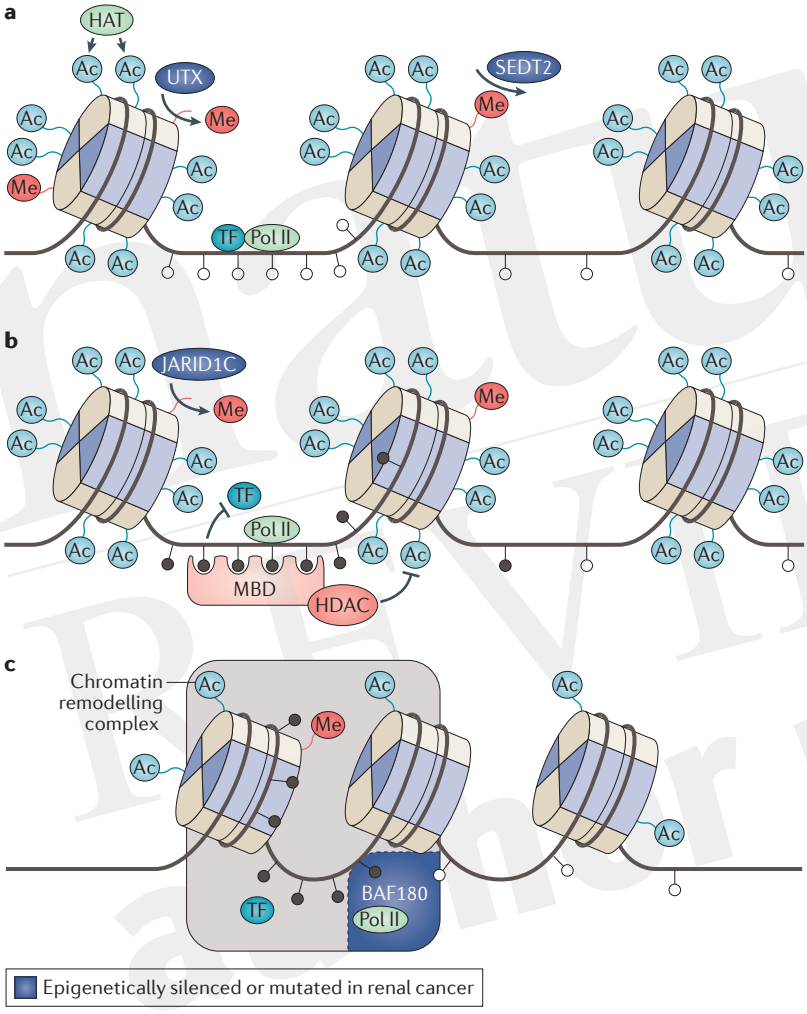

Figure 2 | **Histone acetylation regulates gene expression. a** | Histone acetyltransferases (HAT) acetylate lysine tails; these marks keep the chromatin in an decondensed state, enabling the access of transcription factors (TF) and polymerases (Pol II) to the gene promoters. Proteins such as histone-lysine N-methyltransferase SETD2 and histone demethylase UTX, which are encoded by *SETD2* and *KDM6C* (genes that are mutated in renal cancers), also maintain other open chromatin marks by removal (UTX) or addition (SETD2) of methyl groups to specific lysines. **b** | Following CpG methylation, methyl-CpG-binding domain proteins (MBD) bind to the methylated DNA and recruit histone deacetylases (HDAC), which remove acetyl groups. Additionally, the lysine-specific demethylase 5C (JARID1C) encoded by *KDM5C*, which is mutated in renal cancers, removes specific methyl groups in transcriptionally silenced regions of the genome. **c** | Following deacetylation (and other histone modifications) chromatin remodelling complexes are recruited to the region; these complexes bring nucleosomes together, causing DNA condensation and thus preventing access of TFs or polymerases to promoters.

Frequently dysregulated pathways

WNT–β-catenin, MET and SLIT-2–ROBO1 pathways. The proto-oncogene *CTNNB1*, which encodes β-catenin, is located on chromosome 3p (3p22.1), which is often deleted in RCC and includes several other genes that carry point mutations or are silenced by promoter methylation in RCC and other cancers[4,71] **[Au: Refs OK?]**. β-Catenin is the downstream target of the Wnt pathway, which promotes the expression of tumourigenesis-inducing proteins such as myc proto-oncogene protein (c-MYC) and cyclin D1 (REF. 72). In the absence of binding of WNT to members of the Frizzled (FZ) receptor family or following the binding of slit homologue 2 protein (SLIT-2) to roundabout guidance receptor 1 (ROBO1), glycogen synthase kinase-3 β (GSK-3β) phosphorylates β-catenin. This phosphorylation primes β-catenin for ubiquitylation by jade family PHD finger 1 (JADE-1), a process that depends on the binding of JADE-1 to pVHL[73]. Indeed, the degradation of β-catenin is one of the key HIF-independent functions of pVHL[73]. The binding of WNT to FZ, low-density lipoprotein receptor-related protein (LRP) 5 and LRP-6 recruits GSK-3β, leading to the accumulation of β-catenin and its translocation to the nucleus. In the nucleus, β-catenin acts as a co-activator for Wnt-responsive genes such as *c-Myc* and *cyclin D1*, which promote proliferation, survival and invasion[72].

The Wnt/β-catenin pathway is frequently disrupted in RCC by promoter methylation of key pathway regulators (FIG. 3a), and unregulated β-catenin activation increases the tumourgenicity of renal cells[74]. In addition, *VHL* is inactivated in ~11–30% of ccRCC and pRCC by promoter methylation[75–77] or by loss of chromosome 3. In the absence of pVHL, β-catenin can be targeted for degradation by the E3 ligase F-box/WD repeat-containing protein 1A (β-TrCP, also called β-transducin repeat containing protein)[72,73,78]. This process is often impaired in RCC owing to the inactivation of several Wnt pathway inhibitors by promoter methylation.

Two classes of inhibitor proteins regulate Wnt signalling: the secreted frizzled-related proteins (SFRPs), which bind directly to WNT and prevent it from binding to FZ, and the Dickkopf-related proteins (DKK), which bind to the LRP-5 and LRP-6 component of the WNT receptor complex. In addition, insulin-like growth factor-binding proteins (IGFBP) 1, 2, 4 and 6 bind to LRP-5, LRP-6 and FZ, which inhibits their activation by WNT[79] (FIG. 3a). *SFRP1* (47%), *SFRP2* (53%), *SFRP4* (53%), *SFRP5* (57%), *WIF1* (73%) and *DKK1* (44%), *DKK2* (58%) and *DKK3* (50%) are frequently methylated in RCC[3]. Loss of expression of these inhibitory proteins in RCC results in the accumulation of β-catenin and upregualtion of its target genes[80–84]. *IGFBP1* is also frequently silenced by methylation in ccRCC (35% of tumours) and pRCC (20% of tumours)[85]. Moreover, analysis of data generated by the TCGA Kidney Renal Clear Cell Carcinoma (KIRC) project identified *SFRP1* methylation as marker of poor patient survival[86].

In RCC, 20% and 25% of tumours have reduced expression levels and associated promoter methylation of *ROBO1* and *SLIT-2*, respectively[87,88]. SLIT-2 and hepatocyte growth factor (HGF), and their respective

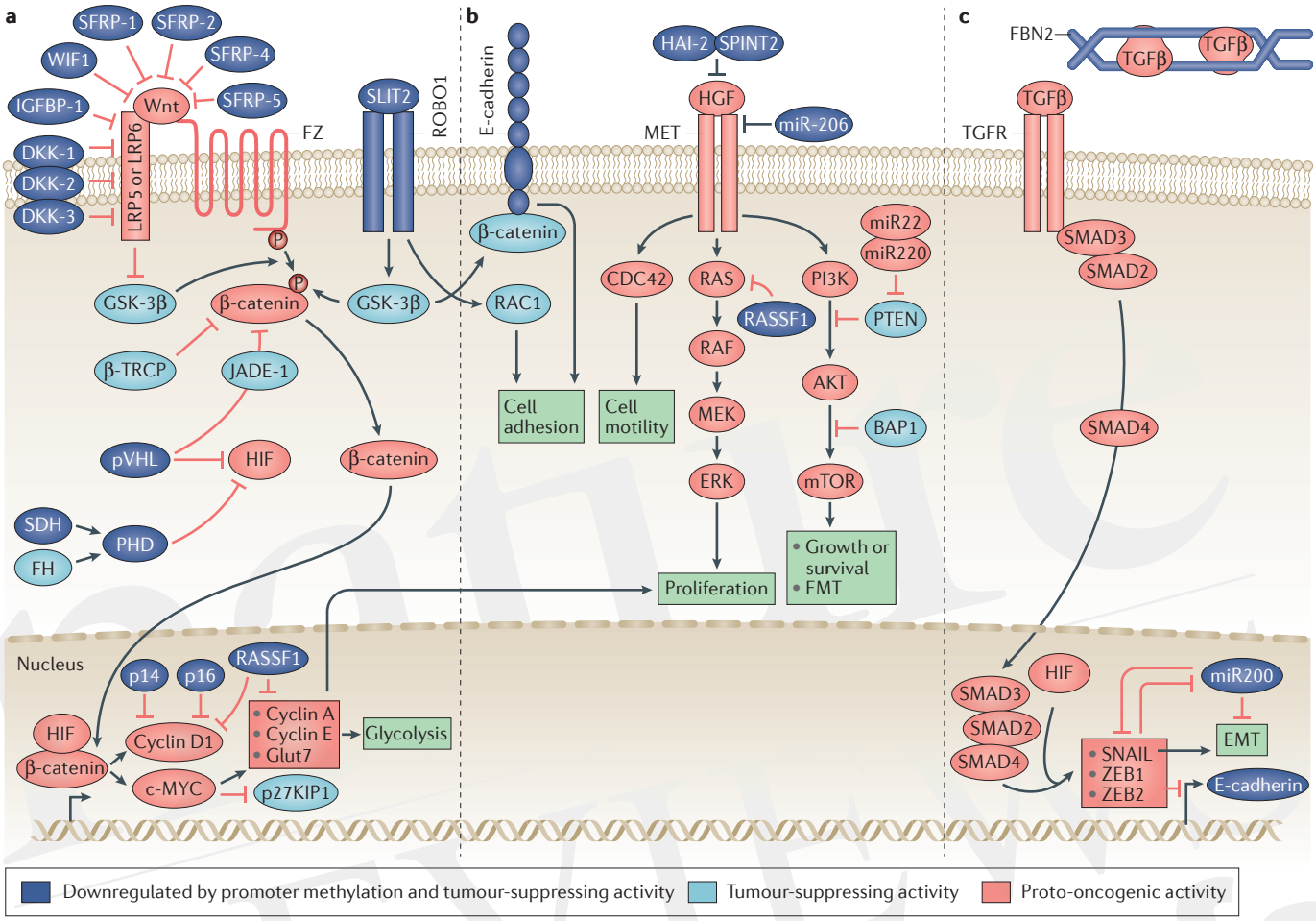

Figure 3 | **Multiple pathways are epigenetically dysregulated in renal cancer. a** | The binding of Wnt to the frizzled receptor (FZ) and LRP5 or LRP6 inhibits GSK-3β, which prevents the phosphorylation of β-catenin. Unphosporylated β-catenin is translocated to the nucleus where it promotes the expression of proto-oncogenic targets such as cyclin D1 and c-MYC. c-MYC upregulates the expression of positive regulators of cell proliferation and glycolysis. The secreted frizzled proteins (SFRP) 1, 2, 4 and 5, Wnt inhibitor factor 1 (WIF1, which inhibit Wnt), Dickkopf (DKK) 1, 2 and 3 and insulin-like growth factor binding protein 1 (IGFBP1, which inhibits LRP5 and LRP6), are frequently downregulated by promoter methylation, resulting in upregulation of β-catenin targets. Von Hippel-Lindau disease tumour suppressor (pVHL) and jade family PHD finger protein 1 (JADE-1) target β-catenin for degradation by ubiquitylating it. Binding of slit homologue 2 protein (SLIT-2) to roundabout guidance receptor 1 (ROBO1) activates GSK-3β and stabilizes the binding of β-catenin to E-cadherin. SLIT-2 and ROBO1 are silenced by promoter methylation in RCC. **b** | E-cadherin, a regulator of cell adhesion and maintenance of the epithelial phenotype, is frequently methylated in RCC. Tyrosine-protein kinase Met (MET, also known as hepatocyte growth factor receptor) is activated by HGF (hepatocyte growth factor), which is inhibited by kunitz-type protease inhibitor 2 (SPINT2, also called HAI-2). The expression of SPINT2 is frequently lost in papillary renal cell carcinoma (RCC) and clear cell RCC. MET expression can be downregulated by miR-206, which is frequently downregulated in RCC. Ras association domain-containing protein 1 (RASSF1) has dual tumour-suppressing functions: it inhibits GTPase HRas (H-RAS-1) signalling and inhibits the accumulation of cyclins A and D1. **c** | Binding of transforming growth factor β (TGFβ) to its receptor activates mothers against decapentaplegic homologue (SMAD) proteins, which upregulate the expression of transcriptional repressors including zinc finger protein SNAI1, zinc finger E-box-binding homeobox (ZEB) 1, and ZEB2. These proteins inhibit multiple targets and promote epithelial-to-mesenchymal transition (EMT) and the expression of several proteins such as E-cadherin. Fibrillin 2 (FBN2), an extracellular matrix protein that is one of the most frequently silenced proteins by promoter methylation in RCC, prevents TGFβ from binding to its receptor and its silencing in RCC leads to ectopic activation of the TGFβ pathway.

receptors ROBO1 and MET (also known as HGF receptor)[89], regulate β-catenin levels. SLIT-2 binds to ROBO1, which activates GSK-3β and results in the degradation of free cytoplasmic β-catenin[90]. Activation of the SLIT-2–ROBO1 pathway also stabilizes the interaction of β-catenin and E-cadherin, which positively regulates cell adhesion[91] and inhibits CDC42, which induces cell adhesion by potentiating RAC1 and inhibiting HGF–MET-induced motility and invasion[92] (FIG. 3b).

Somatic mutations of *RASSF1*, which maps to 3p21.31 —a region of frequent allele loss in many cancers[93] — are rare in RCC; however, silencing by biallelic promoter methylation or by methylation after loss of chromosome 3 are common as *RASSF1* is methylated in 29–91% of tumours[3,94–96]. Ras association domain-containing protein 1, which is encoded by *RASSF1*, functions as a tumour suppressor by regulating metaphase and G1/S phase cyclin degradation

as well as activating apoptotic pathways in response to DNA damage via the ATM or JNK pathways[96] (FIG. 3b).

Activating mutations of *MET* are associated with hereditary pRCC[97] and sporadic pRCC[5,98] but they are rare in sporadic ccRCC[4]. MET is central to the control of several key tumourigenic processes such as cellular proliferation, metabolism and cell motility via the PI3K/AKT/mTOR and the RAS/RAF pathways[99]. *SPINT2* encodes Kunitz-type protease inhibitor 2 (SPINT2, also called HAI-2), which inhibits HGF activator, a protein required for HGF-mediated activation of MET[100] (FIG. 3b). *SPINT2* is frequently methylated and silenced in ccRCC (30%) and pRCC (45%)[101]. These findings suggest that MET-mediated signalling is commonly disrupted in RCC.

Cell adhesion and EMT. In renal cells, pVHL contributes to maintaining the expression of *CDH1*, which encodes E-cadherin, a protein required to preserve renal epithelial morphology[102]. Loss of pVHL causes HIF-induced expression of Zinc finger E-box-binding homeobox 2 (ZEB2, also called SIP1) and zinc finger protein SNAIl, which repress the expression of multiple target genes, including *CDH1* (REFS 103,104). Reduced E-cadherin levels increase tumourigenicity and promote epithelial-to-mesenchymal transition (EMT)[105]. Multiple studies found that E-cadherin is silenced by *CDH1* promoter methylation in ccRCC and pRCC[3].

FBN2, which encodes fibrillin-2, is frequently methylated in RCC, and loss of *FBN2* expression *in vitro* is associated with increased tumourigenicity of RCC cells[8]. Analysis of data from the TCGA KIRC project suggests that *FBN2* methylation is the most common epigenetic mark in RCC as it was present in 40–53% of the 200 patients analysed, and *FBN2* mutations are also fairly frequent (3% of patients)[86]. Fibrillin-2 can sequester transforming growth factor β (TGFβ) in the extracellular matrix and interrupt its downstream signalling pathway, which inhibits E-cadherin expression through SNAI1 **[Au: OK?]**, ZEB1 and ZEB2 (REFS 106,107) (FIG. 3c). This TGFβ-induced inhibition of E-cadherin expression results in enhanced tumourigenicity and EMT[106,107].

Two studies showed that *PCDH8*, a second member of the cadherin family, is also silenced by methylation in ccRCC (19%–58%)[8,86]. This epigenetic mark correlated with poor patient survival[86]. Although the function of *PCDH8* has yet to be elucidated, increasing evidence suggests that this class of adhesion molecules has important roles in cell signalling, cell adhesion and maintenance of the epithelial phenotype[108].

In addition to the extracellular matrix protein fibrillin-2, four members of the collagen family are frequently downregulated or silenced in RCC by promoter methylation: *COL1A1*, (65% in ccRCC and 40% in pRCC)[85], *COL1A2* (29% in ccRCC)[109], *COL14A1* (44% in ccRCC)[110] and *COL15A1* (53% in ccRCC)[110]. *LOXL1*, which encodes lysyl oxidase homologue 1, an extracellular enzyme involved in crosslinking collagens and elastin[111], is also methylated in 35% of patients with ccRCC[110]. How the loss of these proteins is involved in RCC development is unknown; however, extracellular matrix remodelling is clearly emerging as a process central to malignancy and metastasis[112].

Energy homeostasis. *VHL, MET, FLCN, TSC1, TSC2, FH* and *SDH*, which are commonly mutated in kidney cancer, are involved in the cellular response to metabolic stress or nutrient stimulation[113,114]. Consequently, kidney cancer can be defined as a metabolic disease. As described above, some of these genes (*VHL*, *SDHB* and *FLCN*) are also silenced by promoter methylation, although somewhat infrequently[3]. In addition, the complex signalling networks involved in metabolism are commonly altered in RCC owing to epigenetic silencing of component genes. Protein kinase AMP-activated catalytic subunit α1, a master regulator of cellular energy homeostasis encoded by *AMPK*, is a component of the MET–FLCN–mTOR signalling network[115], which can be dysregulated by silencing of *FLCN*[116,117] or *SPINT2* (REF. 101).

Other notable metabolic targets that are epigenetically dysregulated in RCC include *CDO1* (REF. 118) and *SLC16A3* (also known as *MCT4*)[119]. *SLC16A3* encodes solute carrier family 16 member 3, a monocarboxylate transporter that is essential for transport of lactate across the plasma membrane, thus maintaining cellular pH and the appropriate progression of glycolysis[119]. Overexpression of *SLC16A3* correlates with poor patient prognosis and is associated with lower promoter methylation in the tumour compared to less aggressive tumours and normal tissue[119]. CDO1, which is an essential member of the taurine biosynthetic pathway, is involved in the oxidative stress response[120,121]. As in other types of malignancies such as breast cancer[122], colorectal cancer[123] and prostate cancer[124], methylation of the *CDO1* promoter is associated with poor survival of patients with RCC[118]. **[Au: OK?]**

Other pathways. In addition to the signalling pathways outlined above, which are altered by the silencing of multiple genes in RCC, other pathways are affected by the silencing of genes such as *APC*, *APAF1*, *BNC1*, *CASP8*, *CDKN2A*, *FHIT*, *GREM1*, *MGMT*, *TU3A* and *UCHL1* (REFS 3–5,86). For some of these genes, whether and how the absence of the proteins that they encode is involved in RCC development is not yet clearly understood. In some cases, methylation-induced gene silencing is also likely to be a passenger event; however, the silencing of genes through methylation of their promoter in RCC clearly has a widespread influence on tumour progression, and often leads to the dysregulation of key cellular processes such as the cell cycle (*CDKN2A, CDKN2B*[4,6,125]), apoptosis (*CASP8*, *DAPK*[126,127]), genomic stability (*MGMT*[3,125]) and angiogenesis (TIMP3 (REFS 128–130)).

Non-promoter DNA methylation

Most methylation analyses have focused on CpG methylation at defined promoter regions directly adjacent to transcription start site. **[Au: OK?]** However, large-scale genome-wide analysis projects such as ENCODE[131,132] and FANTOM5 (REF. 133) showed that distant regulatory

enhancers also undergo CpG methylation. Enhancer methylation in RCC has been analysed at the genome scale, and one study in particular identified an enrichment of aberrant enhancer methylation associated with networks involved in the cellular response to hypoxia[134]. This study also found that methylation of enhancers correlated with poor prognosis. Improved mapping of enhancer elements and the development of new methylation arrays that include probes for these regions[135] have opened many avenues of investigation still to be explored in this field.

Histone modification in renal cancer

Basic principles

Nucleosomes are composed of approximately 146bp of DNA that are wrapped around an octamer composed of two of each histone protein (H2A, H2B, H3 and H4)[136]. Histones have many chemically modified amino acids; the lysine residues in the external 'tails' can be acetylated, methylated or ubiquitylated, serine residues can be phosphorylated and arginine residues can be methylated[136]. These modifications form the basis of 'the histone code' (REF. 137) and control the expression of associated genes[131]. Chromatin remodelling protein complexes (SWI/SNF and BAF-associated complexes) can be recruited to either condense chromatin and silence a gene region or decondense chromatin and enable gene expression according to the different histone marks. Chromatin remodelling is ATP-dependent and occurs through the physical movement or removal of nucleosomes[138]. *PBRM1* encodes the chromatin-targeting subunit protein polybromo-1 (also called BAF180) of the SWI/SNF chromatin-remodelling complex[139,140]. This multimeric complex has essential roles in DNA repair, proliferation and differentiation[141], and is considered a master regulator of gene expression as it associates with a large number of transcription factors[4,141].

Histone modification in RCC

Until a few years ago, renal tumours were considered to be somewhat unusual as they had a fairly low frequency of gene mutation, with the exception of mutations in VHL. The application of parallel, second-generation sequencing in large-scale projects identified novel and frequent mutations in chromatin remodelling genes such as *PBRM1*, *BAP1*, *SETD2* (although their methylation is uncommon[142]), *KDM5A* (also known as *JARID1C*) and *KDM6A* (also known as *UTX*), which both encode histone demethylases [4,12–14,143].

PBRM1. *PBRM1* is frequently mutated in RCC (41% in ccRCC[14]) and it is likely to be a tumour suppressor gene. Most *PBRM1* mutations are inactivating and loss of expression is associated with an increase in cell proliferation and migration[14], whereas its reintroduction reduces cell proliferation and is associated with G1 cell cycle arrest induced by an increase in $p21^{CIP1}$ (REFS 144,145). Reports of *PBRM1* expression levels in RCC and of its association with poor patient survival are contradictory[146,147]. The precise mechanisms that underlie the oncogenic contribution of *PBRM1* mutations in RCC remain to be clarified; however, in addition to its role in regulating genes that control proliferation, such as $p21^{CIP1}$, *PBRM1* also regulates the expression of cell-adhesion and cell-signalling molecules such as E-cadherin[148–150] (and as such, the subcellular distribution of β-catenin) as well as sister chromatid cohesion[151]. Thus, correct expression of *PBRM1* is likely to have important roles in pathways that are frequently dysregulated in cancer and in maintenance of genomic integrity, which is a barrier to tumourigenicity[152].

SETD2. *SETD2* and its splice variants encode enzymes that depose trimethylated histone H3 lysine 36 marks[153]. *SETD2* is mutated in non-renal tumours[154–156], and is biallelically inactivated in 3–12% of RCCs[4,12,13,143,157]. *SETD2* mutations are associated with genome-wide loss of non-promoter DNA methylation in RCC[4], and loss of *SETD2* is sufficient to reduce the levels of the histone mark H3K36Me3 across the genome[158]. This histone methylation is associated with open heterochromatin and reduced CpG methylation[159,158], and changes in heterochromatin structure (such as nucleosome rearrangement) can alter the accessibility of the spliceosome machinery to genes and alter the expression of splice-variants. *SETD2* mutations could, therefore, change the expression levels and functional structure of many genes[160]. H3K36Me3 is required for serine-protein kinase ATM and TP53-mediated DNA damage checkpoint activation[161] and the recruitment of the DNA mismatch repair protein Msh2 (also called hMutSα)[162], suggesting that loss of *SETD2* function combined with *PBRM1* mutations could increase genomic instability and prevent correct cell-cycle checkpoint control. The potential importance of the tumour-supressing activity of *SETD2* is supported by reduced survival in patients with tumours that harbour *SETD2* mutations[4,157].

KDM5C and KDM6A. The histone demethylases JARID1C and UTX, which are encoded by *KDM5C* and *KDM6A*, are mutated in ccRCC (7% and 1% of patients respectively) [4,12–15,143] and pRCC (1% and 4% of patients, respectively)[5] **[Au: Refs OK?]**. JARID1C removes methyl groups from lysine 4 of histone H3 (H3K4Me3) and UTX removes methyl groups from lysine 27 of histone H3 (H3K27Me3)[163].

HIF induces the expression of JARID1C, which inhibits its target genes by removing H3K4Me3 — a known marker of actively transcribed chromatin[164]. Some of the genes inhibited by JARID1C are HIF-responsive (such as *IGFBP3*, *DNAJC12*, and *COL6A1*)[165], suggesting that in this context, JARID1C acts as a buffer by regulating the level of HIF targets. Uncontrolled upregulation of these HIF targets results in changes in normal cellular physiology, notably, cellular metabolism and angiogenic signalling[166]. A xenograft study showed that *KDM5C* knockdown in $VHL^{-/-}$ cell lines increased tumour growth, supporting the tumour-suppressor function of JARID1C[165]. The full array of genes targeted by JARID1C is unknown but, as H3K4Me3 is a common mark of transcriptional activity, this enzyme is suspected to influence the expression of a large number of genes.

The H3K27Me3 histone mark is associated with repressed transcription[164], suggesting that loss of functional *UTX* in RCC might lead to pathological downregulation of numerous genes. This downregulation has been shown to occur *in vitro*[12].The precise mechanism or relative contribution of these fairly rare mutations to the oncogenic process in the kidney is, however, yet to be elucidated.

BAP1. *BAP1* encodes a deubiquitylating enzyme with multiple targets. This gene is mutated in up to 11% of patients with ccRCC[143,167,168] and 3% of patients with pRCC[5]. *BAP1* is associated with multiprotein complexes that include breast cancer type 1 susceptibility protein (BRCA1) and BRCA1-associated RING domain protein 1 (BARD1). These complexes regulate key cellular pathways including DNA damage response, cell cycle control and apoptosis[169]. Binding of BAP1 to BRCA1 and BARD1 prevents them from ubiquitylating histone H2A lysine 119 (H2A119K)[169]. This ubiquitylation is essential for the tumour-suppressing function of BRCA1 in response to DNA damage, and for the radiation-induced loss **[Au:OK?]** of BAP1-sensitized cells[170].

In addition to its essential role in the orchestrated regulation of the BRCA1-mediated DNA damage response, BAP1 directly deubiquinates H2A119K and binds to E2F family member transcription factors and E2F-responsive promoters. Loss of BAP1 reduces transcription of E2F targets and slows cell-cycle progression[167,171]. The fact that loss of BAP1 slows cell growth is initially counterintuitive as it conflicts with its tumourigenic effect; however, loss of BAP1 also reduces the fidelity of the G1/S phase checkpoint, thus facilitating uncontrolled cell growth[172].

Further work is required to elucidate the mechanisms by which BAP1 loss promotes RCC tumourigenesis; however, several studies showed that BAP1 loss is associated with high tumour grade[167], high tumour stage[173] and poor prognosis[4,157,173,174]. Of note, loss of BAP1 expression correlates with mTORC1 activation[167]. Activation of the mTOR pathway is associated with aggressive tumours and, as described above, this pathway disrupted in hereditary and sporadic renal tumours by direct mutation or epigenetic silencing of pathway regulators (FIG. 3b).

miRNAs in renal cancer

Approximately 3,000 human miRNAs have been experimentally validated as physiologically relevant thus far[175]. This number has risen year-on-year since the discovery of miRNAs and is expected to continue to increase[11], reflecting the physiological importance of these noncoding mRNAs. mRNAs (primary miRNAs) are cleaved by ribonuclease 3, which is encoded by *DROSHA*, into a 60–70-nucleotide hairpin precursor miRNA (pre-miRNA)[176], which is then exported to the cytoplasm where endoribonuclease DICER, another RNase III enzyme, processes it to the final mature 22-nucleotide-long double-stranded miRNA[176]. The mature miRNA directs the miRNA-induced silencing complex (miRISC) to the target mRNA, which leads to its degradation[176].

Dysregulation of both miRNA expression and processing has a key influence on tumour formation[176–178]. In the past 10 years, screens of miRNA expression in RCC have shown widespread miRNA dysregulation[179–181]. Of note, more miRNAs were downregulated or silenced in renal tumours than in normal tissue, and this downregulation or silencing **[Au: OK?]** was often associated with promoter methylation or copy number changes[179,182–188]. Many of these miRNAs target components of networks that are dysregulated by mutations or promoter methylation. These networks include HIF target genes and members of the TGFβ signalling pathway.

Dysregulated cellular pathways

The VHL–HIF network. The two HIF proteins (HIF1α and HIF2α) have contradictory roles in renal tumour progression: HIF1α has tumour-suppressing activity, whereas HIF2α is oncogenic[189–191]. pVHL decreases HIF1α and HIF2α protein levels[22] and induces the expression of miR-30c-2-3p and miR-30a-3p, which target HIF2α transcripts for degradation. Loss of pVHL, therefore, increases HIF1α and HIF2α protein levels and promotes HIF2α transcription through the loss of miR-30c-2-3p and miR-30a-3p[192]. Patients with RCC who have reduced levels of these miRNAs have reduced survival[192].

Both HIF1α and HIF2α upregulate the expression of miR-210 (REF. 193), which in turn targets HIF1α, thus amplifying the oncogenic imbalance between HIF1α and HIF2α[194,195]. miR-210 also targets several genes involved in the hypoxic response, such as genes with roles in the DNA damage response (*RAD52*), angiogenesis (*EFNA3, PTP1B),* cell cycle (*PLK1, CDC25B, Cyclin F, BUB1B, FAM83D, E2F2*) and metabolism (*SDHD*, *ISCU1/2*)[194] (FIG. 4). **[Au: OK?]**

Vascular endothelial growth factor (VEGF) is a regulator of angiogenesis and a key transcriptional target of HIF. Protein levels of VEGF are controlled by miR-206 and miR-106a-5p, which are frequently downregulated in ccRCC[196]. The VEGF receptor can also be upregulated if the levels of miRNAs 206, 106-5a, 216b, 3065-5p, 335-5p or 3065- 5p[196] are decreased. The dysregulation of multiple miRNAs is a common hallmark of cancers, and is often associated with genome-wide methylation and chromatin pattern changes or mutations in miRNA-processing proteins[43,180,197]. High levels of VEGF, which can be secreted by RCC cells, promote the development of new blood vessels that associate with the tumour, and can also act in an autocrine manner to increase tumour cell migration and invasiveness[198].

TGFβ and EMT. The miR-200 family (which includes miR-141, 200a, 200b and 200c) is among the most frequently downregulated group of miRNAs in RCC[199–203]. This miRNA family and miR-30c are involved in the regulation of EMT[199–203]. Their expression can be inhibited by TGFβ signalling, leading to reduced expression of ZEB1, ZEB2 and SIP1 (REFS 199,200,202), which transcriptionally repress E-cadherin and induce EMT[106,107] (FIG. 3c). As discussed above, E-cadherin is essential to maintain epithelial identity and localizes β-catenin to

the cytoplasm. Therefore, loss of expression of these miRNAs can contribute to EMT in the absence of external signals by indirectly reducing E-cadherin levels in the cell. In addition, a 2014 study showed that miR-141 suppressed metastasis by targeting EphA2 transcripts, which reduced cell adhesion by dysregulating AKT, RAC1 and MMP2 pathways[204].

MET and mTOR pathways. miR-206 is frequently downregulated in ccRCC[196] and directly downregulates *c-MET* and *BCL2* transcripts in lung and rhabdomyosarcoma tumours[205,206], suggesting that this mechanism might contribute to tumour progression in RCC. Phosphatase and tensin homologue (PTEN) inhibits PI3K-mediated phosphorylation of AKT and subsequent activation of mTORC1. PTEN is targeted by several miRNAs, including miR-221, miR-222, miR-22, miR-486, miR-21 and miR-23b-3p[207,208]. This inhibition of PTEN increases mTOR activity resulting in elevated levels of proliferation, invasiveness, and migration of RCC cells[209]. miRNA-mediated regulation of protein levels is an essential layer of control that acts on known molecular pathways and is crucial to maintain the intricacy of cell signalling required for normal cellular physiology.

Mechanisms of miRNA dysregulation

As with other tumour suppressors genes, individual miRNAs are often silenced or downregulated in RCC by promoter methylation[4,210–212] and have single nucleotide polymorphisms (SNPs). These SNPs alter the ability of miRNAs to bind to target sequences and are associated with RCC susceptibility[213,214]. In addition, the genes encoding components of the miRNA biogenesis and processing pathways can also be mutated in RCC. Germ-line and somatic mutations in key components of the miRNA biogenesis and processing pathways are common in Wilms tumours. Mutations in *DROSHA* and its binding partner, *DGCR8* (also known as *PASHA*), which processes miRNAs, occur in approximately 12% and 8% of Wilms tumours, respectively[18,38,39]. DICER, the ribonuclease III enzyme responsible for the final maturation of pre-miRNAs, is mutated in ~4% of Wilms tumours[18,38,39,215]; however, mutations in these genes occur infrequently in adult, non-Wilms RCC[4,5].

Loss of these processing enzymes results in widespread, yet distinct patterns of mature miRNA expression. Of note, the expression of the LET-7 family of tumour-suppressing miRNAs is particularly impaired[38,39]. The LET-7 family comprises 12 members, the expression of which is coordinated during development and high in differentiated cells[216]. LET-7 miRNAs inhibit the expression of *C-MYC*[217] and *RAS*[218], which are pro-proliferative proto-oncogenes; *IGF1R, INSR,* and *IRS2*, which positively regulate glucose metabolism and activate the PI3K/mTOR pathway[219]; and *SAL14, Oct4, Sox2, Nanog* and *LIN28*, which induce pluripotency[217,220]. Thus, the LET-7 miRNAs have a pivotal role in determining cell fate and ensuring appropriate cell differentiation. The post-transcriptional levels of LET-7 miRNAs are regulated by *LIN28*, which is overexpressed in multiple types of tumours including Wilms, and the expression of which correlates with tumour aggressiveness[221]. Moreover, *LIN28* overexpression induces Wilms tumours in mice[222].

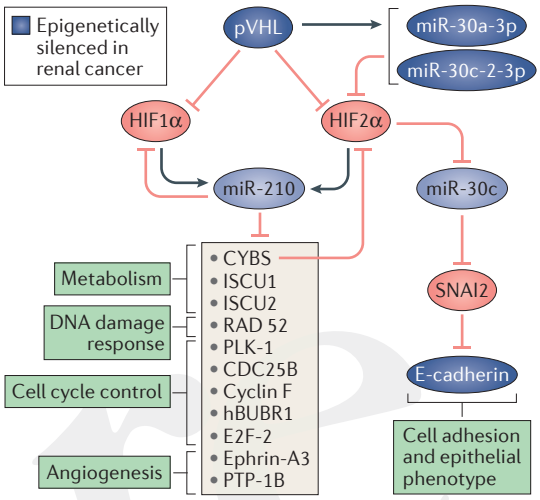

Figure 4 | **microRNAs control the balance of tumour-suppressor HIF1α and oncogenic HIF2.** Loss of von Hippel-Lindau disease tumour suppressor protein (pVHL) upregulates the expression of hypoxia inducible factor (HIF) 1α and HIF2α and downregulates the expression of miR-30a-3p and miR-30c-2-3p, which are HIF2α inhibitors. This loss results in an imbalanced increase in the levels of proto-oncogenic HIF2α. This imbalance is reinforced by the HIF-depended upregulation of miR-210, which reduces HIF1α transcripts levels. miR-210 targets key proto-oncogenic pathways as part of the hypoxic response. Independently of HIF1α, high levels of HIF2α reduce cell adhesion and epithelial-to-mesenchymal transition (EMT) by inhibiting miR-30c, which results in increased expression levels of zinc finger protein SNAI2 (also known as SLUG) and, consequently, reduced E-cadherin levels.

LIN-28 targets LET-7 pre-miRNAs for degradation by polyuridylating them at their 3′ end, which targets them for degradation by the DIS3-like exonuclease 2 (DIS3L2)[223,224] (FIG. 5). Mutations in DIS3L2 cause Perlman syndrome, a rare congenital overgrowth syndrome characterized by the development of Wilms tumours[40]. Loss of DIS3L2 results in an increase in polyuridylated LET-7 and the upregulation of many mRNAs[223–225]; however, the precise influence of DIS3L2 loss on the levels or activity of mature LET-7 miRNAs is yet to be clarified[223,226]. Mutations (exonic deletions) of *DIS3L2* have been identified in 30% of sporadic Wilms tumour[40]. These findings highlight the importance of miRNA regulation in the development of tumours and the requirement for an appropriate balance of expression in the LIN-28–LET7 axis.

Clinical applications

Diagnosis

Early diagnosis of RCC can be challenging, with symptoms often presenting only late in disease progression. Ideally, tumours should be detected early, while they are still spatially confined. Epigenetic marks, in

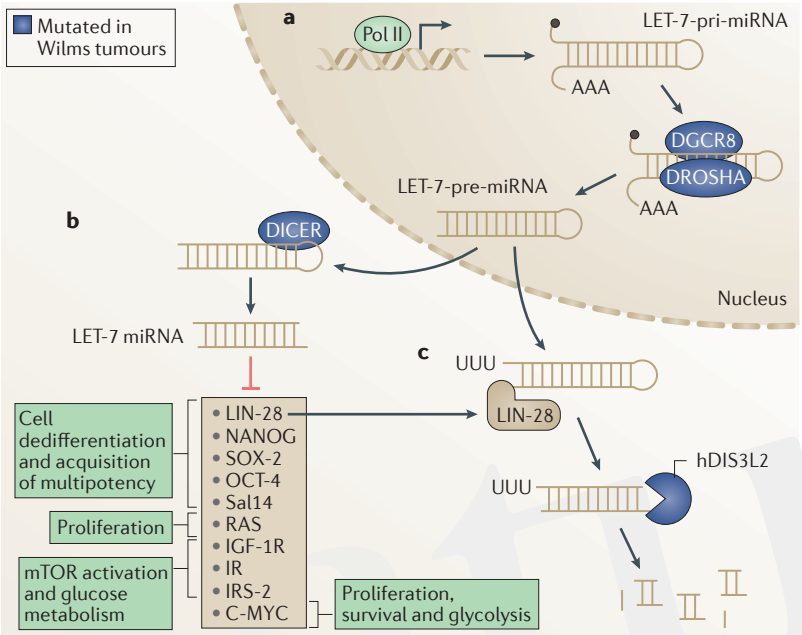

Figure 5 | **microRNA pathways dysregulated in Wilms tumours.** The expression of the LET-7 family of micro RNAs (miRNAs) is dysregulated in Wilms tumours via three mechanisms. **a** | Mutations in *DGCR8* and *DROSHA* prevent processing of pri-miRNAs (removal of poly-A tail and cap) and pre-miRNA formation. **b** | Mutations in *DICER* prevent the processing of LET-7 pre-miRNA into mature miRNAs. **c** | Overexpression of protein lin-28 homologue A (LIN-28A) results in polyuridylation of pre-LET-7 miRNAs and targeted degradation by the DIS3-like exonuclease 2 (hDIS3L2). hDIS3L2 is also commonly mutated in Wilms tumours, resulting in an accumulation of uridylated pre-LET-7 miRNAs. Loss of LET-7 miRNA results in the dysregulation of multiple proto-oncogenic pathways. Pol II, RNA polymerase II.

particular DNA CpG methylation, offer great potential as a diagnostic and prognostic indicator of RCC development. The DNA assays to identify such marks are generally robust and promoter methylation is fairly uniform across the promoter region, which provides a clear advantage over targeted sequencing of candidate mutation sites. Moreover, tumours produce individual cells that can be isolated in urine and plasma, along with cell-free DNA. Several studies have now shown that specific gene methylation patterns can be identified in urine and serum. Gene aberrations identified using such analyses include tumour-specific methylation of *VHL*, *RASSF1*, *MGMT*, *GSTP1*, *p16INK4*, *p14ARF*, *APC*, *TIMP3*, *KILLIN*, *LINE-1* (REFS 227–231), *SFRP1* and several other antagonists of the Wnt/β-catenin pathway[232]. The ability to identify altered methylation of genes such as *VHL* and *RASSF1* in urine and blood is promising as they are silenced early and frequently in tumour evolution, thus providing the potential for the development of an effective diagnostic screen.

Several studies have investigated the prognostic potential of DNA methylation in renal cancers in the past 10 years. Analysis of genome-wide epigenetic profiles of different renal tumour types has identified methylation signatures (epi-signatures) that can identify specific subtypes of RCC[233].

Prognosis

Specific CpG island methylation profiles have been associated with overall patient survival. For example, in a study of nearly 1,000 patients, the methylation of five CpG sites (associated with the genes *PITX1*, *FOXE3*, *TWF2*, *EHBP1L1* and *RIN1)* were negatively associated with overall survival[234].

The metastatic potential of the primary tumour is a key factor that can predict patient survival. Epigenetic changes, which depend on the type of treatment and the tissue-specific microenvironment, are likely to drive much of the metastatic process. Metastasising tumours undergo branched evolution at a genetic level, resulting in many cases where the metastasis is no longer genetically identical to the primary tumour[235,236]; however, genome-wide methylation analyses have revealed that primary renal tumours and resulting metastases remain remarkably similar at the level of DNA methylation[237]. Persistent similarities between primary and metastatic tumours might present good opportunities for the development of novel therapies. Whether specific epigenetic differences exist between primary tumours that readily metastasize and those that do not will be of great interest.

The heterogeneity of mutations within tumours, which results in different subclones that evolve differently, is a major obstacle to clinical translation[235,236,238,239]. Intratumour heterogeneity of DNA methylation might, however, not be as pronounced as intratumour heterogeneity of genomic changes. **[Au: OK?]** In the study that identified five CpG sites that are associated with poor survival[234], the researchers also analysed the presence of these five CpG sites in three separate regions dissected from the same tumour. This analysis, which included tumours from 23 patients, found generally consistent methylation levels within individual tumours. These findings are consistent with the observation that primary and metastatic tumours remain epigenetically similar[237].

Several studies have analysed the presence of multiple miRNAs in serum from patients with RCC to provide prognostic information and identify renal-specific cancers[240–242]. The use of miRNA profiles to predict treatment response has also been proposed and two studies have identified panels of miRNAs that are associated with different responses to sunitinib, an FDA-approved treatment for RCC[243,244].

Treatment development

The identification of miRNAs that regulate multiple pathways central to renal tumour progression presents opportunities for the development of new therapeutic strategies, such as the reintroduction of tumour-suppressing miRNAs that are downregulated in RCC or the inhibition of oncogenic miRNAs. Preclinical trials involving the delivery of miRNA mimics that replace the function of lost miRNAs or of RNA oligonucleotides that act as decoy targets for oncogenic miRNAs have shown promising results in several tumour types. **[Au: Please reference]** *In vitro*, miRNAs have been extremely successful at inducing cell cycle arrest, apoptosis and inhibiting invasion, whereas local delivery of miRNAs *in vivo* can reduce tumour size[245]. However, many challenges

still need to be overcome before this type of molecular therapy can be applied to the clinic. Notably, the development of accurate tumour-specific delivery is a key hurdle to overcome to ensure the success of these therapies.

Several signalling networks are disrupted by promoter methylation and this mechanism is often responsible for the silencing of multiple regulatory genes within the same tumour cell. Targeting promoter methylation might, therefore, be a good therapeutic strategy. Demethylating agents and DNA methyltransferase inhibitors such as azacitidine or decitabine induce global genomic demethylation and have been successfully used in the treatment of haematological neoplasias such as acute myeloid leukaemia and chronic myelomonocytic leukaemia[7]. A preclinical assessment showed that fairly low doses of decitabine consistently reduced proliferation in15 RCC cell lines[246] and several clinical trials are ongoing to determine the efficacy of such treatments in solid tumours[247,248]. However, no large-scale trial for demethylation treatment in RCC is currently underway. Trials of HDAC inhibitors in patients with advanced RCC are currently underway[249–251]. HDAC inhibitors might be particularly effective in patients with *BAP1* mutations as, in addition to reversing gene silencing initiated by aberrant promoter methylation, HDAC inhibitors might also have the ability to reverse H2A ubiquitylation, which is associated with loss of *BAP1* (REF. 250). The identification of mutations in histone-modifying enzymes such as BAP1 and PBRM1, which modulate multiple cell signalling pathways (for example mTOR, p53 and pRB–E2F) that are commonly dysregulated in RCC, provides the opportunity for the development of novel therapies. These new treatments might simultaneously interfere with multiple oncogenic signalling pathways. Moreover, the characterization of mutations and epigenetic marks in these genes, combined with the identification of specific epigenetic biomarkers of RCC, will inform future therapeutic development and personalized treatment for renal cancers.

Conclusions

The study of renal cancer biology over the past 15 years has identified a central role for altered epigenetic control of gene expression in tumour development and progression. We anticipate that, with the development of increasingly detailed analysis tools such as methylation-specific microarrays, epigenome sequencing and RNA sequencing, the integrated analysis of epigenetic regulatory networks will elucidate novel, clinically targetable mechanisms of RCC development. Such mechanistic advances will, hopefully, improve patient outcomes.

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Author contributions

All authors contributed to researching data for the article, discussion of the article's content, writing, and review or editing of the manuscript before submission. **[Au:OK?]**

Competing interests statement

The authors declare no competing interests.

DATABASES

Catalogue of Somatic Mutations in Cancer (COSMIC): http://cancer.sanger.ac.uk/cosmic

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Author biographies

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ToC blurb

000 The epigenetic landscape of renal cancer

Mark R. Morris and Farida Latif

New data suggests that, in addition to mutations in tumour-suppressor genes, renal cancer is associated with epigenetic aberrations. Here, the authors discuss the mechanisms by which epigenetically silenced genes and mutations in genes that are involved in histone modification or chromatin remodelling dysregulate crucial cellular pathways in renal cancer.